

## **IN THE CLAIMS**

This listing of claims will replace all prior versions, and listings, of claims in the application:

1-33. (Canceled)

34. (Currently amended) An *in vitro* assay method for detecting cancer cell growth stimulation by a substance of interest, the method comprising:

maintaining a predetermined population of steroid hormone-responsive cancer cells in a steroid hormone-free nutrient medium comprising a basal nutrient fluid devoid of unbound Fe (III) and comprising calcium ions and an amount of at least one immunoglobulin chosen from the group consisting of non-monomeric plasma IgA and polymeric IgM isolated secreted immunoglobulins that inhibit steroid hormones sufficient to inhibit cell growth in the absence of an inhibition-reversing amount of said a steroid hormone, said cells also being steroid hormone dependent for proliferation *in vivo* when implanted into a suitable host;

adding said substance of interest to said cells and nutrient medium to yield a test mixture;

incubating said test mixture for a predetermined period of time under cell growth promoting conditions; and

determining the cell population in said test mixture after said predetermined period of time, a measurable increase in said cell population indicating a steroid hormone-dependent cancer cell growth stimulating effect by said substance of interest.

35. (Original) The assay method of claim 34 comprising maintaining serum-free assay conditions.

36. (Currently Amended) The assay method of claim 34 comprising adding wherein the nutrient medium further includes steroid-hormone depleted serum to said nutrient medium.

37. (Currently Amended) The assay method of claim 34 wherein the nutrient medium further includes further comprising the step of adding non-heat inactivated serum.

38. (Currently Amended) The assay method of claim 34 wherein said immunoglobulin ~~inhibitor comprises at least one secretory immunoglobulin chosen from the group consisting of dimeric/polymeric IgA, is~~ polymeric IgM and IgG.

39-40. (Canceled)

41. (Currently Amended) The assay method of claim 34 wherein said substance of interest is suspected of containing proteolytic activity, ~~the method further comprising selecting an in which said immunoglobulin inhibitor of a steroid hormone that resists protease degradation.~~

42. (Currently Amended) The assay method of claim 34 wherein said selected immunoglobulin is non-monomeric plasma IgA inhibitor comprises IgA2.

43. (Currently Amended) The assay method of claim 34 further comprising:  
maintaining a second predetermined population of said steroid hormone-responsive cancer cells in a steroid hormone-free nutrient medium ~~comprising a quantity of inactivated immunoglobulin inhibitor that is incapable of inhibiting cell growth~~, said cells also being steroid hormone responsive for proliferation *in vivo* when implanted into a suitable host;

adding said substance of interest to said cells and nutrient medium, to yield a control-test mixture;

incubating said control-test mixture for a predetermined period of time under cell growth promoting conditions;

determining the cell population in said control-test mixture after said predetermined period of time, a measurable increase in said cell population indicating a

control level cell growth stimulating effect by said substance of interest ~~in the presence of said quantity of inactivated immunoglobulin inhibitor.~~

44. (Withdrawn) A method of detecting a steroid hormone antagonistic substance comprising:

maintaining a predetermined population of steroid hormone responsive cancer cells in a nutrient medium comprising a basal nutrient fluid devoid of unbound Fe(III) and comprising calcium ions and comprising a quantity of an immunoglobulin inhibitor of a steroid hormone comprising secreted immunoglobulins sufficient to inhibit cell growth in the absence of an inhibition-reversing amount of said steroid hormone, said cells also being steroid hormone responsive for *in vivo* proliferation;

adding a defined amount of said substance of interest to said cells and medium;

adding to said cells and medium a defined amount of steroid hormone sufficient to stimulate cell growth in the presence of said inhibitor and in the absence of said substance of interest, to yield a test culture;

incubating said test culture for a predetermined period of time under cell growth promoting conditions;

testing said substance of interest for cytotoxic effects on said cells; and

determining the cell population in said test culture after said predetermined period of time, a lack of measurable increase in said cell population not attributable to cytotoxic effects of said substance indicating a steroid hormone antagonistic effect by said substance of interest.

45-94. (Cancelled)

95. (Currently amended) The method of claim 34 comprising:

maintaining a predetermined population of estrogen responsive cancer cells in a steroid hormone-free nutrient medium comprising a quantity of an amount of at least one immunoglobulin chosen from the group consisting of non-monomeric plasma IgA and polymeric IgM of immunoglobulin inhibitor of a steroid hormone sufficient to inhibit

cancer growth in the absence of an inhibition-reversing amount of estrogen, said cell also being estrogen responsive for proliferation *in vivo* when implanted into a suitable host;

adding a defined amount of said substance of interest to said cells and medium, to yield a test culture;

incubating said test culture for a predetermined period of time under cell growth promoting conditions; and

determining the cell population in said test culture after said predetermined period of time, a measurable increase in said cell population indicating ~~estrogen-dependent~~ cell growth stimulating effect by said substance of interest, whereby an estrogenic substance is detected.

96. (Withdrawn) The method of claim 95 further comprising testing said substance of interest for binding to estrogen receptor gamma.

97. (Original) The method of claim 95 further comprising testing said substance of interest for cytotoxic effects on said cells.

98. (Withdrawn) The method of claim 95 further comprising selecting estrogen responsive cancer cells containing estrogen receptor gamma.

99-109. (Canceled)

110. (Currently amended) The method of claim 34 wherein said nutrient medium comprises a Fe (III) chelating agent.

111. (Currently amended) The method of claim 34 wherein said nutrient medium comprises a cell attachment promoting protein.

112. (Currently amended) The method of claim 34 wherein said nutrient medium contains about 1-50 mM calcium ion.

113. (Previously Presented) The method of claim 34 wherein said basal nutrient fluid comprises D-MEM/F-12.

114. (Currently amended) The method of claim 34 wherein said nutrient medium comprises 100 ng/mL to 10  $\mu$ g/mL insulin, 0.3 - 10 nM triiodothyronine, 2 - 50  $\mu$ g/mL diferric transferrin, 5 - 100  $\mu$ M ethanolamine, 0.2 - 5.0 mg/mL bovine serum albumin (BSA), 5 - 20 ng/mL selenium, 2 - 10  $\mu$ M deferoxamine, and, optionally, at least one component chosen from the group consisting of 1 - 50 ng/mL EGF, 0.2 - 20 ng/mL aFGF, 5 - 50  $\mu$ M phosphoethanolamine, 50 - 500  $\mu$ g/mL linoleic acid-BSA, 1 - 50  $\mu$ g/mL reduced glutathione, 0.5 - 2.0 mM glutamine, 1 - 10  $\mu$ g/mL heparin, and 20 - 50  $\mu$ g (~~per 35 mm diameter culture dish~~) human fibronectin.

115-122. (Canceled)

123. (New) The assay method of claim 34 wherein the steroid hormone-responsive cancer cells are selected from the group consisting of MTW9/PL2 rat mammary cells, GH4C1, GH1 and GH3 rat pituitary cells, ZR-75-1 human breast cancer cells, MCF-7A and MCF-K human breast cancer cells, T47D human breast cancer cells, H-301 Syrian hamster kidney cells, LNCaP human prostate cancer cells, and HT-29 human colon cancer cells.

124. (New) The assay method of claim 34 wherein the steroid hormone is selected from the group consisting of Estrogens, Androgens, Progesterone, Glucocorticoids, and Thyroid Hormones.

125. (New) The assay method of claim 124 wherein the steroid hormone-responsive cancer cells are selected from the group consisting of MTW9/PL2 rat mammary cells, GH4C1, GH1 and GH3 rat pituitary cells, ZR-75-1 human breast cancer cells, MCF-7A and MCF-K human breast cancer cells, T47D human breast cancer cells, H-301 Syrian hamster kidney cells, LNCaP human prostate cancer cells, and HT-29 human colon cancer cells.

126. (New) The assay method of claim 36 wherein the steroid hormone-responsive cancer cells are selected from the group consisting of MTW9/PL2 rat mammary cells, GH4C1, GH1 and GH3 rat pituitary cells, ZR-75-1 human breast cancer cells, MCF-7A and MCF-K human breast cancer cells, T47D human breast cancer cells, H-301 Syrian hamster kidney cells, LNCaP human prostate cancer cells, and HT-29 human colon cancer cells.

127. (New) The assay method of claim 36 wherein the steroid hormone is selected from the group consisting of Estrogens, Androgens, Progesterone, Glucocorticoids, and Thyroid Hormones.

128. (New) The assay method of claim 127 wherein the steroid hormone-responsive cancer cells are selected from the group consisting of MTW9/PL2 rat mammary cells, GH4C1, GH1 and GH3 rat pituitary cells, ZR-75-1 human breast cancer cells, MCF-7A and MCF-K human breast cancer cells, T47D human breast cancer cells, H-301 Syrian hamster kidney cells, LNCaP human prostate cancer cells, and HT-29 human colon cancer cells.

129. (New) An *in vitro* assay method for detecting steroid hormone cancer cell growth stimulation by a substance of interest, the method comprising:

maintaining a predetermined population of steroid hormone-responsive cancer cells in a steroid hormone-free nutrient medium comprising a basal nutrient fluid devoid of unbound Fe (III) and comprising calcium ions and an amount of at least one immunoglobulin chosen from the group consisting of non-monomeric plasma IgA and polymeric IgM sufficient to inhibit cell growth in the absence of an inhibition-reversing amount of a steroid hormone, said cells also being steroid hormone dependent for proliferation *in vivo* when implanted into a suitable host;

adding said substance of interest to said cells and medium to yield a test mixture;

incubating said test mixture for a predetermined period of time under cell growth promoting conditions; and

determining the cell population in said test mixture after said predetermined period of time, wherein a measurable increase in said cell population indicates a steroid hormone dependent cancer cell growth stimulating effect by said substance of interest.

130. (New) The method of claim 129 wherein the non-monomeric plasma IgA is dimeric/polymeric IgA.

131. (New) An *in vitro* assay method for detecting estrogenic cancer cell growth stimulation by a substance of interest, the method comprising:

maintaining a predetermined population of estrogen-responsive cancer cells in a steroid hormone-free nutrient medium comprising a basal nutrient fluid devoid of unbound Fe (III) and comprising calcium ions and an amount of at least one immunoglobulin chosen from the group consisting of non-monomeric plasma IgA and polymeric IgM sufficient to inhibit cell growth in the absence of an inhibition-reversing amount of an estrogen, said cells also being estrogen dependent for proliferation *in vivo* when implanted into a suitable host;

adding said substance of interest to said cells and medium to yield a test mixture;

incubating said test mixture for a predetermined period of time under cell growth promoting conditions; and

determining the cell population in said test mixture after said predetermined period of time, wherein a measurable increase in said cell population indicates an estrogenic dependent cancer cell growth stimulating effect by said substance of interest.

132. (New) The method of claim 131 wherein the non-monomeric plasma IgA is dimeric/polymeric IgA.

133. (New) The assay method of claim 36 wherein said steroid hormone-depleted serum is made by a second method comprising:

obtaining a non-heat-inactivated fresh or frozen serum specimen;

performing a first charcoal-dextran extraction on said specimen at about 30-37 °C to yield a first extracted serum; and

performing a second about 30-37°C charcoal-dextran extraction on said first extracted serum to yield the steroid hormone-depleted serum.

134. (New) The second method of claim 133 comprising performing said first charcoal-dextran extraction on said specimen at about 34°C to yield said first extracted serum and performing a second about 34°C charcoal dextran extraction on said first extracted serum to yield said steroid hormone-depleted serum.

135. (New) The assay method of claim 36 wherein said steroid hormone-depleted serum is made by a second method comprising:

obtaining non-heat inactivated fresh or frozen serum and performing an XAD<sup>TM</sup> extraction of said serum to provide a substantially steroid hormone-depleted serum.

136. (New) The method of claim 42 wherein the non-monomeric plasma IgA is dimeric/polymeric IgA.